325 Understanding the Molecular Basis of Trace Amine Receptor Activation by Thyronamines and Related Analogs

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The thyroid hormone is known to increase the contractile function of isolated cardiac myocytes and cause sodium channel and calcium ATPase activation in the heart within seconds to minutes. These rapid effects have been difficult to attribute to the actions of the thyroid hormone receptor due to the slow onset of effects on gene expression. Recently the Scanlan group has discovered that 3-iodothyronamine (T1AM), an endogenous metabolite of the thyroid hormone, can potently activate the G-protein coupled receptor known as the trace amine receptor. When administered to mice, T1AM rapidly induces hypothermia and bradycardia, effects of which are opposite to those observed with thyroid hormone treatment.

A series of thyronamine analogs were synthesized and screened for functional activity to expand the structure activity relationship of thyronamines and to explore the molecular determinants required for trace amine receptor activation by thyronamines.

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326 The $G\alpha_{13}$-RhoA signaling axis is required for SDF-1-induced migration through CXCR4

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The CXC chemokine SDF-1 binds to CXCR4, a seven transmembrane G protein coupled receptor (GPCR) that plays a critical role in many physiological processes that involve cell migration and cell fate decisions, ranging from stem cell homing, angiogenesis, and neuronal development to immune cell trafficking. CXCR4 is also implicated in various pathological conditions, including metastatic spread and HIV infection. Whereas SDF-1 induced migration in CXCR4 expressing cells is sensitive to Pertussis toxin treatment, hence involving heterotrimeric G proteins of the G_i family, whether other G proteins also participate in the chemotactic response to SDF-1 is still unknown. In this study, we took advantage of the potent chemotactic activity of SDF-1 in Jurkat T-cells to examine which heterotrimeric G protein subunits contribute CXCR4-mediated cell migration. We observed that whereas $G\alpha_i$ and $G\beta\gamma$ are involved in SDF-1-induced Rac activation and cell migration, CXCR4 can also stimulate RhoA potently, but independently of G_i. Instead, we found that $G\alpha_{13}$ mediates the activation of RhoA by CXCR4, and that the functional activity of both $G\alpha_{13}$ and RhoA is required for directional cell migration in response to SDF-1. Collectively, our data indicate that signaling by CXCR4 to RhoA through $G\alpha_{13}$ contributes to cell migration when stimulated by SDF-1, thus identifying the $G\alpha_{13}$-RhoA signaling axis as a potential pharmacological target in many human diseases that involve the aberrant function of CXCR4.

327 The identification of *in vivo* phosphorylation sites of M_3-Muscarinic receptor by phospho-peptide mapping

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The muscarinic M_3 acetylcholine receptor (M_3R) is rapidly phosphorylated upon agonist stimulation. We have employed a combination of phospho-peptide mapping, Edman degradation, mass spectrometry and mutagenesis to determine the phospho-acceptor sites amongst the more than 50 putative sites in the M_3R.

Phosphopeptide maps of recombinant M_3 receptors expressed in CHO cells and of receptors endogenously expressed in cerebellar granule neurons allowed us to determine at least fifteen different tryptic phospho-peptides in this receptor, including several that considerably alter their phosphorylation state upon agonist stimulation. Subsequent Edman degradation of the M_3R derived phospho-peptides, followed by phospho-peptide mapping of several single or multiple S⇒A mutants allowed us to identify specific serine residues phosphorylated in the M_3R.

We also compared the maps derived from *in vivo* receptors with those obtained from *in vitro* phosphorylation of M_3R domains with a number of protein kinases to determine if these kinases are involved in the phosphorylation of the M_3R in intact cells. In addition to GRKs, both Casein kinase 1α and Casein kinase 2 are shown to phosphorylate the third intracellular loop of the M_3R.

Our results highlight the enormous complexity of M_3-muscarinic receptor phosphorylation. These data will be discussed in the context of our proposal that differential phosphorylation underlies the receptor specific signalling observed in various physiological tissues

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328 AGE-RELATED BALANCE IMPAIRMENT IN MICE LACKING VG1037, AN ORPHAN GPCR LOCALIZED TO BRAIN MOTOR SYSTEMS

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VG1037 is a Gq-coupled orphan GPCR. Mutant mice were made in which the VG1037 gene (GPR139) was replaced with the reporter gene LacZ. Staining for the LacZ product revealed extensive, but specific, localization of VG1037 to the nervous system, with a preferential localization to motor systems, including lateral striatum, globus pallidus, inferior olivary complex, and the cerebellar deep nuclei. In addition, marked LacZ expression was observed in the vestibular nuclei, spinal cord and dorsal root ganglia. Mice were behaviorally evaluated for motor function using runway and open field gait analyses, rotarod, balance beam, and other tests. At 2 months of age, mice showed no impairment in any of these behaviors. However, starting at 5 months of age, mice lacking the VG1037 gene displayed a significant impairment in balance, manifested as a decreased latency to fall on the rotarod and less movement on the balance beam. In addition, the older null mutants showed significantly abnormal lateral placement of their forepaws when turning corners on the open field, but normal forepaw placement on a straightaway. Therefore, VG1037-null mutants appear to have a primary age-related balance deficit, consistent with the localization of this gene to the motor and vestibular systems.